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Activating cardiac E2F1 induces up-regulation of pyruvate dehydrogenase kinase 4 in mice on a short term of high fat feeding

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ABSTRACT

A high fat diet (HFD) induces substantial cardiac metabolic alteration(s), but the initiating molecular events remain unclear. We assessed the early cardiac energy metabolic changes in C57/BJ mice fed a HFD for 10 days. Carbohydrate oxidation was markedly decreased in mice on a HFD, in which up-regulation of pyruvate dehydrogenase kinase 4 (PDK4) was evident. Concurrently, E2F1, a transcription factor controlling PDK4 expression, was activated, as was cyclin D1, an upstream-molecule of E2F1, and eukaryotic initiation factor 4E (eIF4E), a modulator of cyclinD1 translation. Hence, HFD may initiate early cardiac metabolic alterations through the eIF4E/cyclin D1/E2F1/PDK4 axis.

1. Introduction

A high fat diet (HFD) can lead to insulin resistance and the development of cardiovascular diseases [1]. One of the HFD-induced metabolic alterations in the heart is an increase in fatty acid oxidation and a reduction in glucose utilization [2,3]. Competition between fatty acids and carbohydrates can occur at the level of the initial uptake of substrates into the cells, or at the subsequent transport into the mitochondria. At the level of the mitochondria, the pyruvate dehydrogenase complex (PDC) catalyzes the conversion of pyruvate to acetyl-CoA, thereby controlling glucose oxidation. Activity of PDC can be negatively modulated by pyruvate dehydrogenase kinase 4 (PDK4), that in turn, is regulated at the transcriptional level by multiple factors, including transcription factors, such as E2F1 [4], PPARα [5], PGC-1α [6], Foxo1 [7] and estrogen-related receptors [6].

The transcription factor E2F1, besides being a critical cell cycle regulator, also plays an important role in controlling mitochondrial function [8,9] and glucose homeostasis [4,10,11]. The activity of E2F1 is largely controlled by the phosphorylation of retinoblastoma (Rb), leading to dissociation of the Rb/E2F1 complex thereby releasing E2F1 for nuclear translocation [12,13]. Phosphorylating Rb (p-Rb), at a specific residue serine 780 (s780) by cyclin-dependent kinase 4 (CDK4) [14], requires elevated cytosolic cyclin D1 to form a complex with CDK4 [15]. Up-regulation of cytosolic free cyclin D1 can be regulated at the transcriptional level [16], but frequently occurs as a result of post-translational modifications [17,18]. A specific regulation of cyclin D1 translation by eukaryotic initiation factor 4E (eIF4E) has also been demonstrated [19,20].

Two overlapping binding sites of E2F1 on the PDK4 promoter have been identified [4]. Over-expression of E2F1 in C_2C_{12} myoblasts up-regulates PDK4 expression and suppresses glucose oxidation [4]. Conversely, inhibition of E2F1 prevents the development of myocyte hypertrophy in vitro [21], while loss of E2F1 in vivo blunts PDK4 expression and increases cardiac glucose oxidation [4].

The aim of this study is to explore how the HFD initiates the signalling pathways in mediating the myocardial energy metabolism in mice early in the course of obesity development.

2. Materials and methods

All animals received care and treatment according to Canadian Council on Animal Care and University of Alberta Health Sciences Animal Welfare Committee. Male C57BL/6 mice of 8 week of age were placed on either a HFD (60% calories from lard) or a low fat

Abbreviations: HFD, high fat diet; LFD, low fat diet; PDC, pyruvate dehydrogenase complex; PDK4, pyruvate dehydrogenase kinase 4; Rb, retinoblastoma; PPARα, peroxisome proliferator-activated receptor; PGC-1α, peroxisome proliferator-activated receptor gamma coactivator 1-α; CDK4, cyclin-dependent kinase 4; eIF4E, eukaryotic initiation factor 4E; eIF2α, eukaryotic initiation factor 2α; 4EBP1, eukaryotic initiation factor 4E-binding protein-1

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diet (LFD) (4% calories from lard, Research Diets; D12492) for 10 day or 3 week period. Hearts were excised and immediately frozen in liquid N_2 for biochemical analyses or perfused in the isolated working mode.

2.1. Heart perfusions

Isolated working hearts were perfused with Krebs–Henseleit solution containing 1 mM lactate, 5 mM glucose, 0.8 mM palmitate, 3% fatty acid free bovine serum albumin, and 2.5 mM free Ca^{2+}. To measure fatty acid oxidation and lactate oxidation rates, hearts were subjected to a 60 min perfusion with perfusate containing [9,10-^{3}H]palmitate and [U-^{14}C]lactate. To measure rates of glycolysis and glucose oxidation, another series of hearts were perfusated in Krebs–Henseleit solution containing [5-^{3}H] glucose and [U-^{14}C] glucose. Oxidation and glycolytic rates were determined simultaneously by quantitative collection of $^{14}CO_2$ and ^3H_2O produced by the hearts. The perfusions were performed at a left atrial preload of 11.5 mmHg and an aortic afterload of 50 mmHg. Heart rate, cardiac output, and cardiac work were measured as described previously [22].

2.2. Assessment of β-hydroxylacyl CoA dehydrogenase and citrate synthase activity

The activity of β-hydroxylacyl CoA dehydrogenase and citrate synthase was measured based on the continuous spectrophotometric rate determination, following the reduction of NAD^+ at 340 nm [23] or the increased production of TNB at 412 nm [22], respectively.

2.3. Myocardial triacylglycerol (TG), malonyl-CoA, long chain-CoA, and ceramide determination

Myocardial TG was quantitated colorimetrically with an enzymatic assay (Wako Pure Chemical Industries) [22]. Myocardial malonyl-CoA, long chain-CoA and ceramide were assessed by HPLC [22].

2.4. Pyruvate dehydrogenase complex (PDC) activity

Measurement of PDC was performed using a radioisotopic-coupled enzyme assay following acetyl-CoA production, as previously described [24].

2.5. Antibodies for immunoblot analysis

Antibodies for phospho-Rb(s780), phosphor-cyclin D1(Thr 286)/total cyclin D1, eIF4E, phosphor-4EBP1, phosphor-eIF2α/total eIF2α and lamin A were purchased from Cell Signaling Technology (Beverly, MA). Antibodies for CDK4 and PDK4 were obtained from Abcam, while anti-E2F1 and anti-actin were from Santa Cruz Biotechnology. The immunoreactive bands were quantified by scanning densitometry with ImageJ image analysis software.

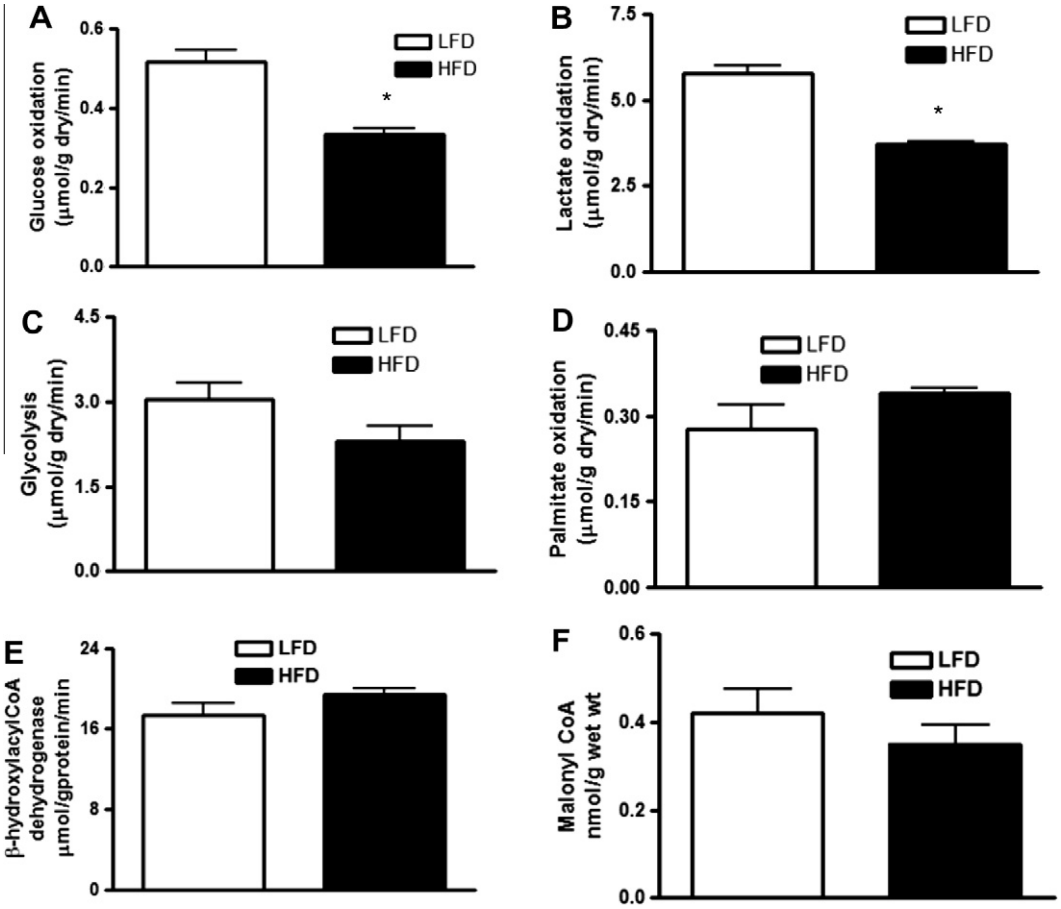

Fig. 1. Altered myocardial energy metabolism in mice fed a HFD for 10 days. In the isolated working hearts, the basal energy metabolism was assessed in the absence of insulin. (A) Rates of glucose oxidation. (B) Rates of lactate oxidation. (C) Rates of glycolysis. (D) Rates of palmitate oxidation. (E) Activity of β-hydroxylacyl CoA dehydrogenase. (F) Myocardial malonyl CoA level. Values represent mean ± S.E.M. ($n = 5$). *$P < 0.05$, significantly different from the LFD.

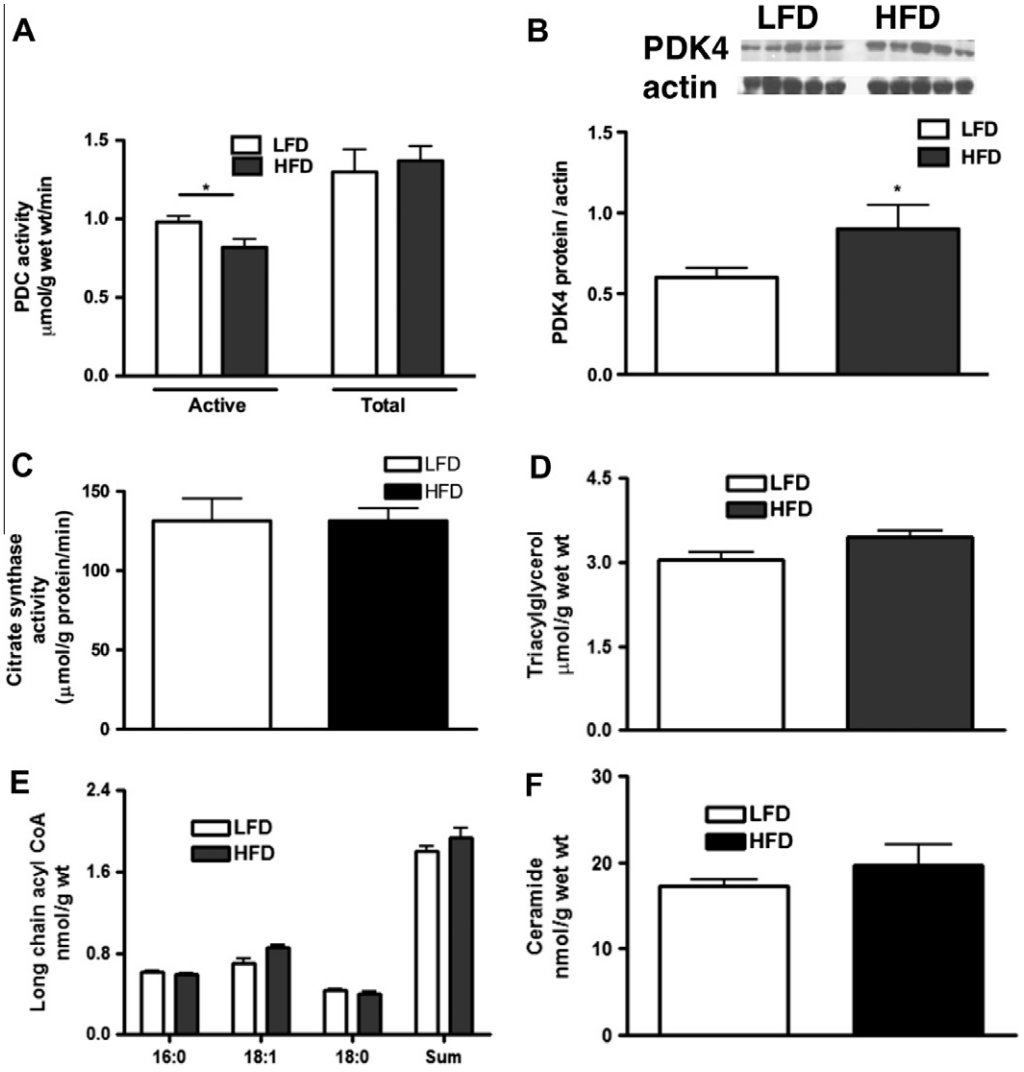

Fig. 2. Effect of HFD on myocardial PDC, PDK4, citrate synthase activity and lipids. (A) Active and total PDH activity. (B) Expression of PDK4. (C) Citrate synthase activity. (D) Levels of triacylglycerol (TG). (E) Levels of long chain acyl CoA. (F) Levels of ceramide. Values represent mean ± S.E.M. ($n = 5$). $^{*}P < 0.05$, significantly different from the LFD.

2.6. Quantitative RT-PCR analysis

Levels of mRNA were quantified by TaqMan Real time RT-PCR using 18S RNA as the internal control. The primers and probes were purchased from Applied Biosystems (ABI) Canada. The primer and probe sequences for mouse CDK4 were designated by using "Primer Express" software from ABI. Forward Primer: GAGGCCTTTGAACATCCCAA. Reverse primer: TCAGTTCGGGAAGTAGCACAGA; Probe: TTGTACGGCTGATGGAT.

2.7. Statistical analysis

All data are presented as the mean ± S.E.M. Statistical analysis of the data was performed with the use of a 2-tailed Student *t*-test to determine the statistical significance of differences between the HFD mice and LFD mice. $P < 0.05$ was considered significant.

3. Results

3.1. Cardiac carbohydrate oxidation rates are reduced in mice subjected to 10 days of a HFD

To examine what early cardiac energy metabolic changes occurred with a HFD, isolated working heart perfusions were performed in mice after 10 days on a HFD. A decrease in the glucose oxidation (Fig. 1A) and lactate oxidation rates (Fig. 1B) was seen in mice subjected to a HFD, compared to LFD controls. In contrast, rates of glycolysis (Fig. 1C) and palmitate oxidation (Fig. 1D) were unaffected. Consistent with the lack of changes in fatty acid oxidation, the activity of β-hydroxylacyl CoA dehydrogenase (Fig. 1E), a key enzyme involved in fatty acid β-oxidation, and the levels of malonyl CoA, a key molecule preventing fatty acid CoA from entering mitochondria (Fig. 1F), were preserved.

3.2. Inactivation of myocardial PDC results from a HFD-induced up-regulation of PDK4 expression

Reduction in glucose uptake or suppression in PDC activity could account for the decrease in carbohydrate oxidation rates. However, the expression of glucose transporter 1 and 4 was unaltered by the diet (data not shown), and glycolytic rates were not significantly decreased. In contrast, the amount of active PDC was significantly decreased in mice fed a HFD compared to LFD controls (Fig. 2A). This occurred despite no differences in the total PDC activity (Fig. 2A), suggesting an enhanced phosphorylation and inhibition of PDC in the HFD mice hearts. This notion is further supported by an enhanced PDK4 protein expression in mice fed a HFD (Fig. 2B). In addition, neither mitochondrial citrate synthase

Table 1
Cardiac function was unaltered in mice fed a HFD for 10 days.

	Control (low fat 4%, $n = 15$)	High fat (60%, $n = 15$)
Cardiac work (ml * mmHg/min)	6.9 ± 0.30	7.0 ± 0.3
Cardiac output (mL/min)	10 ± 0.47	10.2 ± 0.48
Aortic flow (mL/min)	6.7 ± 0.42	6.6 ± 0.47
Cardiac power (mJ/min)	76 ± 3.9	77 ± 3.5

Statistic analysis of the data was performed with a 2-tailed Student *t*-test.

activity (Fig. 2C), the level of various myocardial lipids (Fig. 2D–F), nor cardiac function (Table 1) was altered by the diet. Thus, the HFD-induced early reduction in carbohydrate oxidation rates is not the result of lipid accumulation or mitochondrial and cardiac dysfunction, but a result of suppression of PDC activity due to induction of cardiac PDK4.

3.3. Nuclear translocation of cardiac E2F1 was enhanced concomitant with the induction of PDK4

Consistent with other studies [2], HFD-induced nuclear translocation of PPARα and PGC-1α, known to modulate PDK4, was not observed in mice after a HFD (data not shown), suggesting an alterative pathway(s) involved in the increased myocardial PDK4 transcription. We therefore examined what effect a HFD had on the expression of cardiac E2F1, a newly identified molecule for direct regulating the gene encoding PDK4 [4]. The abundance of nuclear E2F1 protein was significantly enhanced in mice fed a HFD compared to LFD controls (Fig. 3A). This likely occurred as a result of

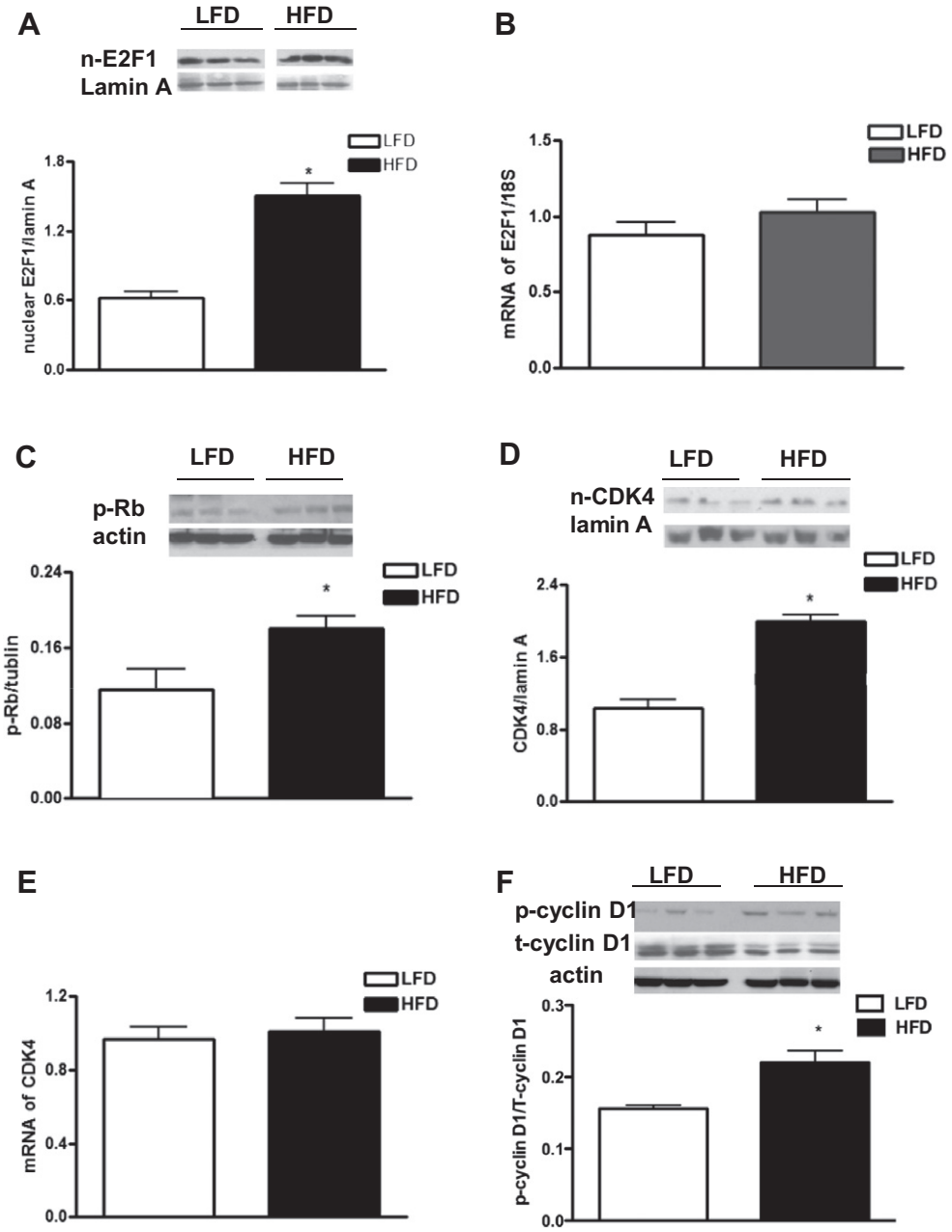

Fig. 3. Effect of HFD on the expression of myocardial E2F1, p-Rb(s780) and CDK4. (A) Expression of E2F1 in the nucleus. (B) Levels of E2F1 mRNA. (C) Phosphorylation of Rb. (D) expression of CDK4 in the nucleus. (E) Expression of CDK4 mRNA. (F) Ratio of p-cyclin D1 at Thr286 to the total cyclin D1 in cytosol. Values represent mean ± S.E.M. ($n = 5$). *$P < 0.05$, significantly different from the LFD.

a HFD-induced nuclear translocation of cardiac E2F1, as the mRNA level of cardiac E2F1 was unaltered (Fig. 3B). This is supported by the up-regulation of p-Rb, at residue serine 780 (s780) (Fig. 3C), a well known indicator for nuclear translocation of E2F1 [12,13]. Consistent with the notion that p-Rb (s780) is a specific target of CDK4 [14], the HFD-induced accumulation of nuclear CDK4 was also observed (Fig. 3D). This appears to be a result of a HFD-induced nuclear translocation, as CDK4 mRNA levels were unaltered by the HFD (Fig. 3E). In addition, it has been demonstrated that nuclear translocation of CDK4 results in an enhanced phosphorylation of cyclin D1(p-cyclin D1) at residue threonine 286 (T286) [25,26]. Indeed, increased phosphorylation of cyclin D1 was seen in the hearts of mice on a HFD compared to LFD controls (Fig. 3F). Thus, the data is consistent with cardiac E2F1 being activated due to a HFD-induced nuclear translocation of CDK4 resulting in a phosphorylation of Rb (s780).

3.4. Nuclear translocation of CDK4 by a HFD is initiated via accelerated translation of cyclin D1

It is known that nuclear translocation of CDK4 occurs when the abundance of cytosolic cyclin D1 is enhanced [27]. We questioned whether HFD initiates this process via a transcriptional or a translational regulation of cyclin D1. In contrast to the unaltered mRNA level of cardiac cyclin D1 (Fig. 4A), the abundance of cytosolic cyclin D1 was decreased (Fig. 4B), concomitant with an increase in the expression of nuclear cyclin D1 (Fig. 4B) in the mice fed a HFD compared to LFD controls. This suggests a translational mechanism underlying HFD-induced regulation of cyclin D1. To further prove this, activation of eukaryotic initiation factor 4E (eIF4E), known to play a specific role in stimulating translation of cyclin D1 [19,20], was assessed by its protein expression and the phosphorylation of eukaryotic initiation factor 4E-binding protein-1 (4EBP1) [28]. A significantly increased phosphorylation of cardiac 4EBP1 was observed in the HFD mice (Fig. 4C and D), despite an unaltered eIF4E protein. In addition, activation of eIF2α kinases can lead to the phosphorylation of 4EBP1 [29]. Similar to p-4EBP1, phosphorylation of eIF2α was also significantly enhanced by the HFD (Fig. 4E). Hence, HFD-induced signals initiate complex formation of cyclin D and CDK4, most likely via accelerating the translation of cyclin D1.

3.5. Up-regulation of PDK4 occurs in conjunction with time-dependent activation of E2F1

To investigate whether the parallel elevation of E2F1 and PDK4 seen in hearts after 10 days of a HFD persists with longer periods of a HFD, the period of HFD was prolonged to 3 weeks. Similar to 10 days of HFD, the nuclear expression of E2F1 was significantly enhanced (3.97 ± 0.45 vs 1.98 ± 0.21 in the LFD, $P < 0.05$). In line with this, the expression of p-Rb (s780) (Fig. 5A) and PDK4 (Fig. 5B) was stepwise elevated in the same fashion after a HFD, concomitant with a decrease in glucose oxidation rates (Fig. 5C). In addition, the upstream molecules involved in accelerating translation of cyclin D1, such as p-eIF4E (Fig. 5D) and p-eIF2α (Fig. 5E), were also time-dependently up-regulated by the short term HFD.

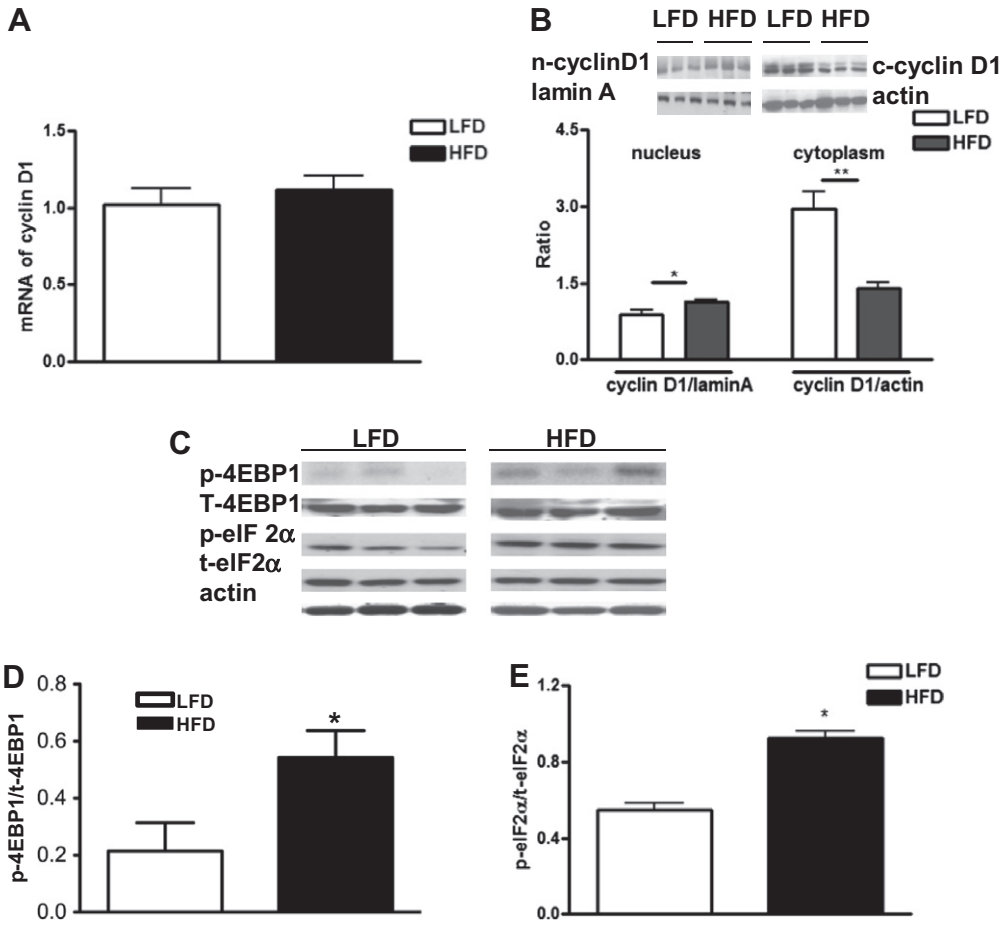

Fig. 4. Effect of HFD on enhancing the abundance of myocardial cyclin D1 and activating eIF4E. (A) Expression of cyclin D1 mRNA. (B) Expression of cyclin D1 in the cytosol and nucleus. (C) Expression of p-4EBP1. (D) Expression of p-eIF2α. Values are mean ± S.E.M., $^{*}P < 0.05$, $^{**}P < 0.01$, significantly different from the LFD.

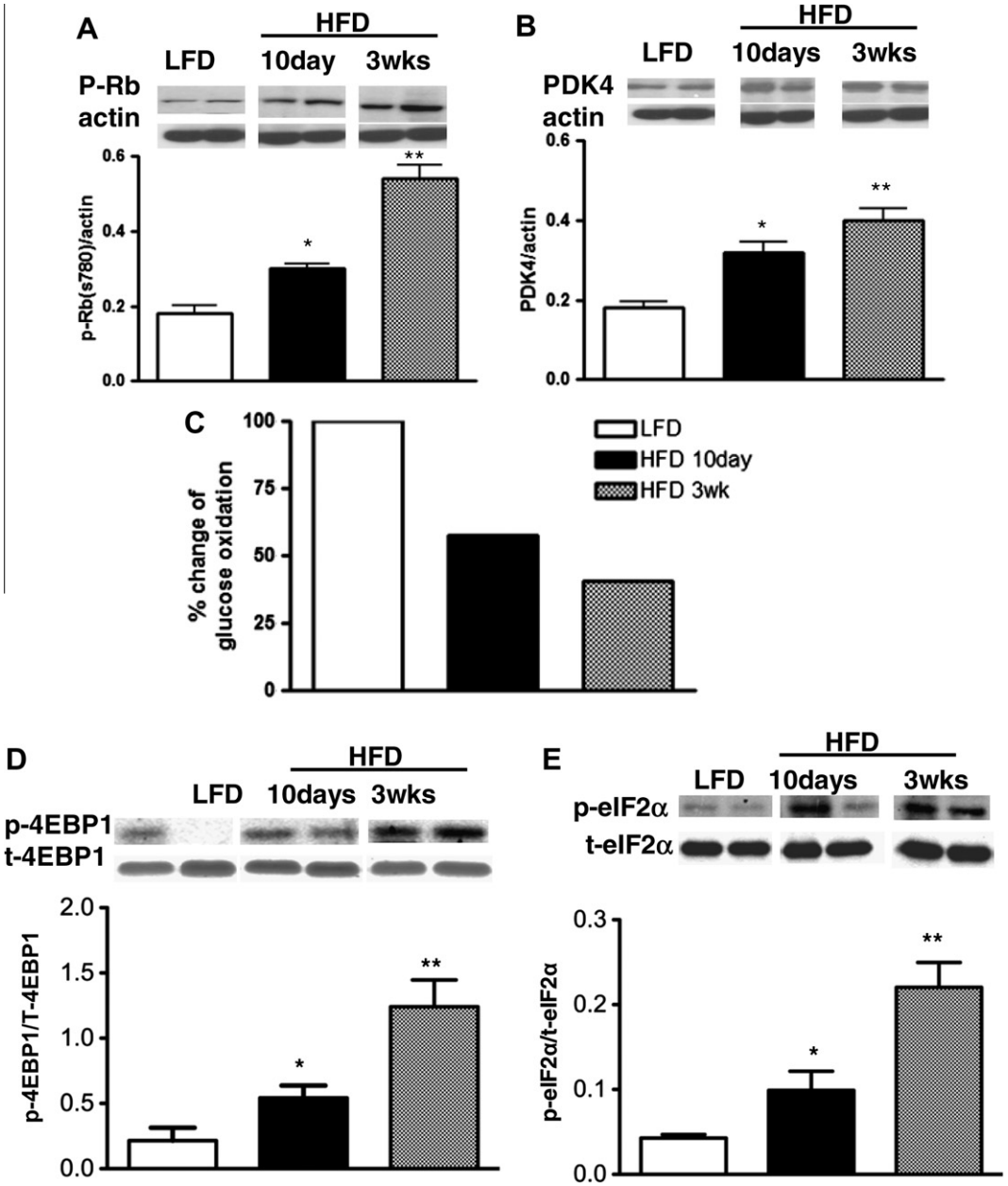

Fig. 5. Alterations of myocardial p-Rb, PDK4 and p-4EBP1 in mice after 10 days and 3 weeks of a HFD. (A) Time dependent up-regulation of p-Rb (s780). (B) Time dependent up-regulation of PDK4. (C) Time-dependent changes of glucose oxidation rates. (D) Time-dependent expression of p-4EBP1. (E) Time-dependent expression of p-eIF2α. Values are mean ± S.E.M., $^{*}P < 0.05$, $^{**}P < 0.01$, significantly different from the LFD.

4. Discussion

This study provides a number of novel findings regarding the effects of a HFD on early alterations in energy metabolism in the heart. This includes: (1) an early and selective decrease in carbohydrate oxidation occurs, (2) cardiac E2F1, known as controlling the gene encoding PDK4, accumulates in the nucleus following 10 days of a HFD, coincident with the up-regulation of PDK4 and down-regulation of PDC activity, (3) HFD-induced nuclear translocation of CDK4 and cyclin D1 is responsible for the nuclear accumulation of E2F1, through CDK4-related phosphorylating Rb thereby releasing E2F1, and (4) nuclear translocation of cyclin D1 occurs due to HFD-induced activation of eukaryotic initiation factors via accelerating translation of cyclin D1. The significance of these findings is that we describe a new mechanism through which HFD-induced activation of cardiac eukaryotic initiation factors may couple cell cycle regulators and PDK4 to control mitochondrial carbohydrate oxidation as an early response to a HFD.

To support the conclusion that myocardial PDK4 is a major player involved in the HFD-induced reduction in carbohydrate oxidation, we demonstrated an early suppression of PDC activity in mice after 10 days of a HFD. The possibility, that membrane distribution of glucose transporters could be a causal factor [2] is ruled out since glycolysis was not depressed, and lactate uptake and oxidation occur independent of glucose transporters. As a result the HFD-induced reduction of cardiac glucose and lactate oxidation rates is likely due to a decreased conversion of pyruvate to acetyl CoA by PDC activity. Thus, the HFD-induced up-regulation of myocardial PDK4 likely represents a critical mechanism for the early decrease in myocardial lactate and glucose oxidation. In addition,

we demonstrate that the level of nuclear E2F1 is significantly elevated. This appears to be the result of a HFD-induced nuclear translocation of E2F1, since E2F1 mRNA level is unaffected by the diet. Instead of determining E2F1 transcriptional activity, we took advantage of the specific antibody raised against p-Rb (s780), based on the fact that up-regulation of p-Rb (s780) is a reliable indicator of E2F1 activation [14,30]. We demonstrate a HFD-induced up-regulation of p-Rb (s780). Furthermore, phosphorylating Rb(s780) occurs in a CDK4-specific manner in vivo [12–14]. To be active, CDK4 needs to form a complex with cyclin D1, in which increased abundance of cytosolic free cyclin D1 is a prerequisite [15]. On the basis of unchanged cyclin D1 mRNA levels, and the specific role of eIF4E in stimulating translation of cyclin D1 [19,20], we demonstrate that the activity of eIF4E, which is largely controlled by phosphorylation of 4EBP1 [28], is enhanced in a time-dependent manner. Hence, the HFD induced signals initiate translocation of E2F1, thereby up-regulating PDK4, most likely through accelerating the translation of cyclin D1 via activating eIF4E.

In current study, we are unable to explore whether decreasing E2F1/p-Rb decreases PDK4 expression in HFD mice. However, genetic manipulations, such as E2F1 knockout (*E2F1*$^{-/-}$) mice, have shown a blunted PDK expression and improved glucose oxidation in isolated hearts [4]. In contrast, exogenous E2F1 over-expression has been shown to up-regulate PDK4 in mouse myoblasts, in IMR90 fibroblasts, and in C2C12 cells [4], while also reducing glucose oxidation in human AC16 cells [31]. In addition, a markedly induced PDK4 expression also occurs in Rb-deficient mouse embryo fibroblasts, which strongly suggests a transcriptional requirement of PDK4 by induction of E2F1 [4]. In addition, we have also observed in angiotensin II-induced hypertrophic mouse hearts that impaired glucose oxidation, due to elevated PDK4 expression, is accompanied by a nuclear accumulation of cardiac E2F1 and an induction in phosphorylated Rb (s780) (Lopaschuk et al. unpublished data). Importantly, reversible regulation of glucose oxidation in AngII-induced hypertrophic mouse heart occurs in conjunction with the down-regulation of PDK4, E2F1 and p-Rb (s780) (Lopaschuk et al. unpublished data). An interesting finding from our study is that the nuclear translocation of PPARα and PGC-1α, known to modulate PDK4 promoter [6,7], was not affected by the diet, whereas the nuclear translocation of E2F1 was enhanced with stepwise increased expression of p-Rb and PDK4 and decreased glucose oxidation after 10 days to 3 week of a HFD. Thus, we propose that the biological relevance of E2F1 in regulating PDK4 is important.

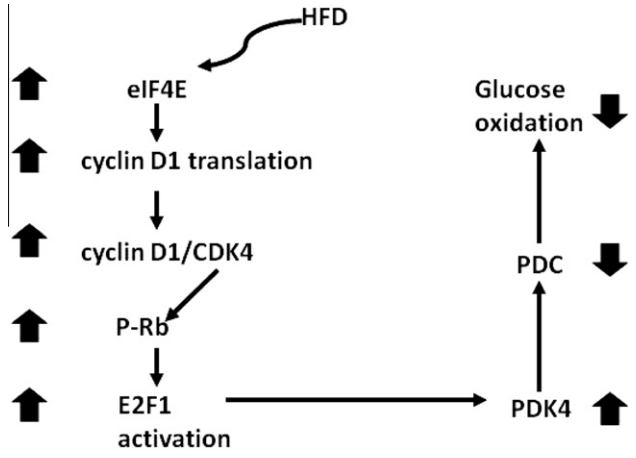

Fig. 6. Proposed pathway for suppressed myocardial carbohydrate oxidation rates by a short term of HFD. HFD-induced signals activates eIF4E to enhance the translation of cytosolic free cyclin D1. As a result, nuclear translocation of cyclin D1 with its catalytic partner CDK4 phosphorylates Rb, thereby releasing E2F1 to the nucleus where up-regulating PDK4 expression occurs. Thus, up-regulation of PDK4 inhibits PDC activity thereby restricting the influx of acetyl CoA into mitochondria and reducing carbohydrates oxidation.

A proposed signaling pathway for short-term HFD-induced down-regulation of carbohydrate oxidation is depicted in Fig. 6. HFD-induced signals enhance the level of cytosolic free cyclin D1, most likely through accelerating translation of cyclin D1 via activating cardiac eIF4E. As a result, nuclear translocation of cyclin D1 with its partner CDK4 phosphorylates Rb at s780, thereby releasing E2F1 to the nucleus, where it up-regulates PDK4 expression. At this point, the function of CDK4 and cyclin D1 is no longer required [15], and nuclear export of cyclin D1 leads to enhanced expression of p-cyclin D1 [15,25]. Our data offers an explanation for the findings that cardiac PDK4 is increased, which is very often one of the proteins that displays the largest changes in expression in response to metabolic disease or conditions that affect cardiac metabolism. A limitation of our study is that we are unable to extend the studies by modulating the key regulatory components such as 4EBP1 or CDK4 in vivo, to further strengthen our conclusions. It is important to emphasize, however, that modulation of E2F1 in hearts might be exploited therapeutically to increase glucose oxidation in obesity and diabetes.

Acknowledgments

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References

[1] Lopaschuk, G.D., Folmes, C.D. and Stanley, W.C. (2007) Cardiac energy metabolism in obesity. Circ. Res. 101, 335–347.
[2] Wright, J.J. et al. (2009) Mechanisms for increased myocardial fatty acid utilization following short-term high-fat feeding. Cardiovasc. Res. 82, 351–360.
[3] Park, S.Y. et al. (2005) Unraveling the temporal pattern of diet-induced insulin resistance in individual organs and cardiac dysfunction in C57BL/6 mice. Diabetes 54, 3530–3540.
[4] Hsieh, M.C., Das, D., Sambandam, N., Zhang, M.Q. and Nahle, Z. (2008) Regulation of the PDK4 isozyme by the Rb-E2F1 complex. J. Biol. Chem. 283, 27410–27417.
[5] Huang, B., Wu, P., Bowker-Kinley, M.M. and Harris, R.A. (2002) Regulation of pyruvate dehydrogenase kinase expression by peroxisome proliferator-activated receptor-alpha ligands, glucocorticoids, and insulin. Diabetes 51, 276–283.
[6] Araki, M. and Motojima, K. (2006) Identification of ERRalpha as a specific partner of PGC-1alpha for the activation of PDK4 gene expression in muscle. FEBS J. 273, 1669–1680.
[7] Furuyama, T., Kitayama, K., Yamashita, H. and Mori, N. (2003) Forkhead transcription factor FOXO1 (FKHR)-dependent induction of PDK4 gene expression in skeletal muscle during energy deprivation. Biochem. J. 375, 365–371.
[8] Cam, H., Balciunaite, E., Blais, A., Spektor, A., Scarpulla, R.C., Young, R., Kluger, Y. and Dynlacht, B.D. (2004) A common set of gene regulatory networks links metabolism and growth inhibition. Mol. Cell 16, 399–411.
[9] Hlaing, M., Spitz, P., Padmanabhan, K., Cabezas, B., Barker, C.S. and Bernstein, H.S. (2004) E2F-1 regulates the expression of a subset of target genes during skeletal myoblast hypertrophy. J. Biol. Chem. 279, 43625–43633.
[10] Fajas, L., Annicotte, J.S., Miard, S., Sarruf, D., Watanabe, M. and Auwerx, J. (2004) Impaired pancreatic growth, beta cell mass, and beta cell function in E2F1 (−/−) mice. J. Clin. Invest. 113, 1288–1295.
[11] Darville, M.I., Antoine, I.V., Mertens-Strijthagen, J.R., Dupriez, V.J. and Rousseau, G.G. (1995) An E2F-dependent late-serum-response promoter in a gene that controls glycolysis. Oncogene 11, 1509–1517.
[12] Martelli, F. and Livingston, D.M. (1999) Regulation of endogenous E2F1 stability by the retinoblastoma family proteins. Proc. Natl. Acad. Sci. USA 96, 2858–2863.
[13] Trouche, D., Le Chalony, C., Muchardt, C., Yaniv, M. and Kouzarides, T. (1997) RB nd hbrm cooperate to repress the activation functions of E2F1. Proc. Natl. Acad. Sci. USA 94, 11268–11273.

[14] Kitagawa, M. et al. (1996) The consensus motif for phosphorylation by cyclin D1-Cdk4 is different from that for phosphorylation by cyclin A/E-Cdk2. EMBO J. 15, 7060–7069.
[15] Morgan, D.O. (1995) Principles of CDK regulation. Nature 374, 131–134.
[16] Albanese, C. et al. (1999) Activation of the cyclin D1 gene by the E1A-associated protein p300 through AP-1 inhibits cellular apoptosis. J. Biol. Chem. 274, 34186–34195.
[17] Muise-Helmericks, R.C., Grimes, H.L., Bellacosa, A., Malstrom, S.E., Tsichlis, P.N. and Rosen, N. (1998) Cyclin D expression is controlled post-transcriptionally via a phosphatidylinositol 3-kinase/Akt-dependent pathway. J. Biol. Chem. 273, 29864–29872.
[18] Russell, A., Thompson, M.A., Hendley, J., Trute, L., Armes, J. and Germain, D. (1999) Cyclin D1 and D3 associate with the SCF complex and are coordinately elevated in breast cancer. Oncogene 18, 1983–1991.
[19] Rosenwald, I.B. et al. (1995) Eukaryotic translation initiation factor 4E regulates expression of cyclin D1 at transcriptional and post-transcriptional levels. J. Biol. Chem. 270, 21176–21180.
[20] Rosenwald, I.B., Lazaris-Karatzas, A., Sonenberg, N. and Schmidt, E.V. (1993) Elevated levels of cyclin D1 protein in response to increased expression of eukaryotic initiation factor 4E. Mol. Cell. Biol. 13, 7358–7363.
[21] Vara, D., Bicknell, K.A., Coxon, C.H. and Brooks, G. (2003) Inhibition of E2F abrogates the development of cardiac myocyte hypertrophy. J. Biol. Chem. 278, 21388–21394.
[22] Zhang, L., Ussher, J.R., Oka, T., Cadete, V.J., Wagg, C. and Lopaschuk, G.D. (2010) Cardiac diacylglycerol accumulation in high fat-fed mice is associated with impaired insulin-stimulated glucose oxidation. Cardiovasc. Res. 89, 148–156.
[23] Cornille, E., Abou-Hamdan, M., Khrestchatisky, M., Nieoullon, A., de Reggi, M. and Gharib, B. (2010) Enhancement of L-3-hydroxybutyryl-CoA dehydrogenase activity and circulating ketone body levels by pantethine. Relevance to dopaminergic injury. BMC Neurosci 11, 51.
[24] Shangraw, R.E., Rabkin, J.M. and Lopaschuk, G.D. (1998) Hepatic pyruvate dehydrogenase activity in humans: effect of cirrhosis, transplantation, and dichloroacetate. Am. J. Physiol. 274, G569–G577.
[25] Diehl, J.A., Zindy, F. and Sherr, C.J. (1997) Inhibition of cyclin D1 phosphorylation on threonine-286 prevents its rapid degradation via the ubiquitin-proteasome pathway. Genes Dev. 11, 957–972.
[26] Alt, J.R., Cleveland, J.L., Hannink, M. and Diehl, J.A. (2000) Phosphorylation-dependent regulation of cyclin D1 nuclear export and cyclin D1-dependent cellular transformation. Genes Dev. 14, 3102–3114.
[27] Tamamori-Adachi, M. et al. (2003) Critical role of cyclin D1 nuclear import in cardiomyocyte proliferation. Circ. Res. 92, e12–e19.
[28] Gingras, A.C., Gygi, S.P., Raught, B., Polakiewicz, R.D., Abraham, R.T., Hoekstra, M.F., Aebersold, R. and Sonenberg, N. (1999) Regulation of 4E-BP1 phosphorylation: a novel two-step mechanism. Genes Dev. 13, 1422–1437.
[29] Kazemi, S. et al. (2007) A novel function of eIF2alpha kinases as inducers of the phosphoinositide-3 kinase signaling pathway. Mol. Biol. Cell 18, 3635–3644.
[30] Trimarchi, J.M. and Lees, J.A. (2002) Sibling rivalry in the E2F family. Nat. Rev. Mol. Cell Biol. 3, 11–20.
[31] Palomer, X., Alvarez-Guardia, D., Davidson, M.M., Chan, T.O., Feldman, A.M. and Vazquez-Carrera, M. (2011) The interplay between NF-kappaB and E2F1 coordinately regulates inflammation and metabolism in human cardiac cells. PLoS One 6, e19724.